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Alpha-fetoprotein : a parameter of fetal development.

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ALPHA-FETOPROTEIN:
A PARAMETER OF FETAL DEVELOPMENT

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YALE

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ALPHA-FETOPROTEIN:

A PARAMETER OF FETAL DEVELOPMENT

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INTRODUCTION

In an effort to insure the birth of healthy infants, obstetricians have been searching for parameters by which they can safely evaluate fetal development. Amniocentesis has provided a valuable tool for studying fetal metabolic products. Of special value in amniocentesis is any element that can be proven to be of fetal origin and can therefore solely reflect fetal growth and development.

A protein specific for the fetus was first described in fetal calf serum by Pedersen[24] in 1944. A similar protein was originally demonstrated in the human fetus by Bergstrand and Czar[5] in 1956. This protein, of molecular weight 70,000, occupied the region of the alpha-globulins during electrophoresis, and was named alpha-fetoprotein (AFP).

Gitlin and Boesman,[11] in 1967, determined the sites of alpha-fetoprotein synthesis in human and rat embryos. They incubated the following tissues with ^{14}C labeled amino acids: brain, heart, intestines, kidney, liver, lung, skeletal muscle, placenta, and skin from the human and rat, and the yolk sac from the rat. Using rabbit antisera to AFP, and immunoelectrophoresis, they detected radioactive AFP only in human and rat liver and rat yolk sac. Placenta and other organ systems were found not to be involved in the synthesis of alpha-fetoprotein.

The function of AFP has not yet been elucidated. Smith[34] reported in 1972 that he was unable to detect any AFP in the sera of newborns

with severe congenital malformations, and he hypothesized that this protein may be necessary for normal organogenesis. He injected rabbit AFP antisera into the yolk sac of chicken eggs. Into a control group he injected non-immune normal rabbit sera. A similar experiment was performed injecting antisera intraperitoneally into pregnant rats.

Congenital abnormalities, failure of development or abortion occurred only in the experimental group. 50% of the chickens developed congenital abnormalities, and 40% of the eggs were dead and undeveloped. 10% of the rats had meningomyeloceles, and 25% of the rat pregnancies terminated in abortion, stillbirth, or maternal mortality. As these results did not occur in the controls, it is unlikely that they are a result of a non-specific antigen-antibody reaction, but rather that alpha-fetoprotein has a physiological role in normal embryogenesis.

Association with Neoplasms

Prior to 1971 and the development of a radioimmunoassay for alpha-fetoprotein,[27] no one had been able to detect AFP levels in normal healthy adults. However, Tatarinov,[35] in 1965, demonstrated the presence of a protein that was immunoelectrophoretically identical to AFP in patients with primary liver cancer.

This finding has been confirmed and expanded by many researchers, and AFP has been detected in some adults with embryonal teratomas

and, rarely, hepatitis.[9,21] The frequency of detectable AFP levels in histologically verified hepatocellular cancer varies from between 38% and 81% in different parts of the world,[8] with marked geographical and racial variation.[16]

AFP in Pregnancy

Whether AFP could be demonstrated in the serum of pregnant women had been a subject of controversy for some years. Foy and associates (1970),[10] using immunodiffusion, found detectable AFP levels in 7 out of 20 pregnant women; all seven of them were more than 30 weeks pregnant. Alpert and Zuckerman (1970),[4] however, examined the sera from 142 pregnant women by electroimmunodiffusion and found no positive reactions.

In 1971, Seppala and Ruoslahti[27] developed a highly sensitive radioimmunoassay for AFP. They were able to detect levels in normal adults that ranged from 1.5-16.5 ng/ml. In examining the sera from 63 women at different stages of pregnancy and after delivery, they demonstrated increasing AFP levels throughout pregnancy;[30] during the first trimester, the range was from 18-119 ng/ml; during the second trimester the concentrations were from 96 to 302 ng/ml; and during the third trimester, the AFP level ranged from 160 to 550 ng/ml. In three out of ten mothers, the concentration one day post-partum was greater than during labor. Maternal levels of AFP were found to fall rapidly during post-partum; the average half-life of maternal AFP was five days.

The question arose as to whether the pregnant women's elevated levels were a result of transplacental passage of alpha-fetoprotein produced by the fetus, or if metabolic changes in pregnancy caused the mother to produce this protein. Foy and associates[10] had found that they could induce baboons to produce elevated levels of AFP by feeding them a pyridoxine deficient diet. Noting that pregnant women and women on oral contraceptives have a similar fall in serum pyridoxine levels, they compared AFP levels in pregnant women and women on oral contraceptives to see if such a vitamin deficiency could result in increased concentrations of AFP. By their immunodiffusion assay, none of the women on oral contraceptives had detectable levels of AFP, whereas 35% of the pregnant women did. In addition, Foy and his co-workers examined the sera from the infants born to the women in the study; 100% of the newborns had detectable levels, suggesting that the AFP levels seen in pregnant women were a result of transplacental passage.

Amniotic and Fetal Levels of AFP

Fetal levels of AFP have been well documented. Gitlin and Boesman (1966)[11] found that fetal synthesis of AFP increases for the first twenty weeks of gestation, but that the concentration in fetal serum peaks at about 3 mg/ml at 13 weeks, and then begins to decrease as the fetus grows more rapidly than the production of AFP. Norgaard-Pederson (1973)[22] found a significant correlation between gestational age and cord

serum AFP concentration; he calculated the 95% confidence limits for estimating gestational age by means of AFP concentration in cord blood to be $\pm$ 16 days.

Seppala and Ruoslahti (1972)[31] showed that gestational age can also be estimated by assaying amniotic fluid AFP. They used radioimmunoassay to determine the levels from 65 amniotic fluid samples. AFP concentration in amniotic fluid was found to decrease from the 14th week of gestation until term. In the second trimester, the AFP levels ranged from 2,800-26,000 ng/ml, and during the third trimester, they ranged from 15-535 ng/ml.

The correlation between gestational age and decreasing AFP concentration in amniotic fluid was statistically highly significant ($p < 0.001$). In addition, they found that all AFP levels in amniotic fluid prior to the 37th week of pregnancy were 185 ng/ml or higher.

The amniotic fluid sample from one infant with congenital nephrosis was markedly elevated to 4,900 ng/ml at 36 weeks gestation. Also, four samples of fetal urine were taken during the second trimester; AFP concentrations ranged from 5,900 to 48,000 ng/ml. These findings suggest that fetal urine may be the major source of amniotic fluid during pregnancy.

AFP in the Diagnosis of CNS Malformations

Brock and Sutcliffe, in 1972,[6] undertook a retrospective study of alpha-fetoprotein in the amniotic fluids of 31 pregnancies leading to anencephaly, three leading to spina bifida, and three resulting in hydrocephaly. In the anencephalic cases, all AFP determinations made between 25 and 35 weeks gestation were markedly elevated to between three and ten times normal levels; after 35 weeks gestation, five out of nine cases of anencephaly had AFP levels two to ten times normal.

In one case of spina bifida in which the fluid was obtained at 13 weeks, AFP concentration was four times the upper limit of normal. Amniotic fluid samples from pregnancies resulting in spina bifida or hydrocephaly that were obtained after 30 weeks gestation did not show any increase in AFP.

Following the experiences of Brock and Sutcliffe, other investigators initiated prospective studies on women who were at risk for having an anencephalic child due to a previous pregnancy resulting in anencephaly. Several investigators reported terminating pregnancies at 18 to 20 weeks gestation when an AFP level of five to twenty times normal was detected. In all cases the aborted fetus had anencephaly[17, 25] or a large myelocele.[2]

Harris and co-workers[14] compared amniotic fluid and maternal serum AFP levels in the early diagnosis of neural tube defects. They found raised amniotic levels in anencephaly and "open" spina bifida

and normal levels in association with closed lesions, including encephalocele and hydrocephalus. Seller's group[28] also examined the serum AFP concentration in seven anencephalic fetuses and found them to be normal for their gestational age. These findings suggest transudation of fetal blood components as the mechanism of the elevated amniotic AFP levels with neural tube defects.

Radioimmunoassay was employed by Harris' group[14] in evaluating maternal serum. Their samples from pregnancies involved in CNS malformations were from the second and third trimesters and yielded results within the normal range in eight out of nine cases, including open spina bifida and anencephaly. The isolated elevated maternal level occurred in the case of a spontaneous abortion of an anencephalic.

Similarly, Seller and co-workers[28] found maternal serum AFP concentrations unreliable in indicating neural tube defects. They concluded that closed neural tube lesions will usually be missed by assaying for AFP, and that maternal concentrations of AFP cannot be relied upon to indicate CNS malformations in early pregnancy.

AFP in Fetal Stress

Alpha-fetoprotein represents a fetal marker in maternal serum as well as in amniotic fluid, and currently the AFP levels in several types of high-risk pregnancies are being documented. Vivian and Ward[37] recently examined maternal AFP levels in Rh-immunized pregnancies.

They found all fifteen of their samples to fall within the normal range. Three samples represented pregnancies which resulted in stillbirths, and in two of those cases the maternal serum was drawn within twenty-four hours before death. They therefore concluded that evaluating maternal AFP concentrations is not helpful in assessing fetal distress in Rh disease.

Seppala and Ruoslahti[32] studied the AFP levels in maternal serum and amniotic fluid in 48 pregnancies complicated by maternal immunization against Rh and ABO systems. In examining the maternal serum, they found the AFP concentrations in the immunized women to be significantly higher than the normal controls; however, in most cases (41 out of 48) the AFP levels were within normal limits. Three samples that were above normal limits represented severe Rh-immunization leading to intrauterine fetal death.

The AFP concentrations in the amniotic fluid samples from Rh disease were found to be uniformly distributed around the normal median. Samples from three cases of severe erythroblastosis, and one sample obtained four days prior to fetal death were all above the normal median. In two of the cases of severe erythroblastosis, the concentrations of AFP were found to increase, instead of decrease, with advancing gestation.

Seppala and Ruoslahti concluded that amniotic and maternal serum AFP concentrations may be elevated in severe Rh-immunization but

usually remain within the wide range of normal limits. They also inferred that amniotic AFP levels may paradoxically rise with gestational age in the face of severe erythroblastosis fetalis.

They speculated that the raised maternal AFP levels in fetal distress may be either primary or secondary to the fetal disease. The elevated AFP may be the result of increased fetal-maternal transfusion, which would boost maternal immunization and lead to more severe disease.

Or possibly, the increased concentration could be secondary to disease; it could reflect higher fetal production of the protein, perhaps as a result of delayed fetal liver maturation due to prolonged stress.

Higa and co-workers[17] compared normal amniotic levels of twenty proteins, including AFP, with samples from pregnancies that resulted in Down's Syndrome, anencephaly, and fetal death. They found that the total protein concentration in amniotic fluid was not useful in antenatal diagnosis of those entities, but that IgM, IgA, IgG and AFP were elevated in cases of anencephaly and fetal death. These findings agreed with those of Seller[29] and Harris;[14] in examining serial samples from cases of anencephaly, they found markedly elevated concentrations of AFP that decreased with gestational age but always remained three to ten times normal.

Higa's group also looked at maternal serum levels of AFP in a case of fetal death and found it to increase with fetal demise:

Gestational Age	Maternal AFP
159 days	72 ng/ml
176	no FHT
177	744 ng/ml
181	1192 ng/ml

Seppala and Ruoslahti (1973)[33] reported the amniotic AFP concentrations in ten abnormal pregnancies. They noted marked elevations in two out of four cases of fetal death, including one in which the amniotic AFP level rose from 400 ng/ml one week prior to death to 48,000 at death.

They described three pregnancies in diabetic women in which the amniotic fluid AFP concentrations showed a small but surprising increase late in gestation. They also noted a slightly elevated concentration in the earlier samples from the diabetics. Seppala and Ruoslahti stated that the increases in AFP were associated with fetal distress, but they failed to elaborate.

Norgaard-Pedersen and Klebe[23] (1974) determined the AFP concentration in cord blood from 45 newborns of diabetic mothers. They found a significantly ($p < 0.05$) higher concentration of AFP in infants of insulin-treated mothers than in gestationally matched normals. The AFP concentration in the five infants of non-insulin dependent diabetics fell within normal limits.

In summary, AFP has been found to be a major serum protein of the fetus. In normals it reaches a maximum serum concentration of

3 mg/ml at 13 weeks gestation. From this level it decreases at a predictable rate until at birth it is approximately 1% of the peak level, and by one year of age, the concentration is less than 0.001% of the peak level.

AFP concentration in the amniotic fluid parallels fetal serum levels of the protein except when malformations result in excessive transudation of the protein. Maternal serum concentrations of AFP, however, increase throughout pregnancy, and rapidly fall during the first postpartum week. Elevated adult levels of AFP have also been demonstrated in association with hepatomas and malignant teratomas.

Extremely elevated concentrations of AFP have been documented in the maternal serum and amniotic fluid in cases of open neural tube defects. Some investigators have reported increased levels with fetal demise and fetal stress in Rh-immunization and diabetes.

The association of massively elevated amniotic AFP levels with neural tube defects is presently used at many centers as evidence for elective termination of affected pregnancies. A radial immunodiffusion assay is employed at this hospital to determine if an at-risk pregnancy is affected with a CNS malformation. It is the purpose of this investigation to examine the use of the radial immunodiffusion assay of AFP in exposing other forms of fetal distress.

MATERIALS AND METHODS

Study Group

82 amniotic fluid samples from 77 pregnancies were studied: 42 from the second trimester and 40 from the third. Amniotic fluid was obtained at the time of cesarean section or by transabdominal amniocentesis and was kept frozen until assayed.

Control samples for the second trimester were obtained from patients undergoing elective abortion for social reasons or a genetic tap that later resulted in a normal pregnancy. Controls for the third trimester of pregnancy came from patients undergoing elective repeat cesarean section or amniocentesis for determination of fetal maturity.

Samples for pregnancies at risk for fetal stress included those with the maternal complications of Rh-immunization, diabetes mellitus, and pre-eclampsia, as well as congenital malformations. Also included in the study were sera collected from eight women at the time of amniocentesis.

Radial-Immunodiffusion

Alpha-fetoprotein was measured by radial immunodiffusion as originally described by Mancini.[20] A monospecific rabbit antiserum and a standard containing a known concentration of AFP were kindly

supplied by Dr. Alexander Baumgarten. The purity of the AFP standard was checked by spectrophotometry, and the concentration of 6.6 micrograms/mililiter was confirmed by an outside laboratory.

Alpha-fetoprotein agarose was prepared using 1 gm. agarose, 0.1 gm. sodium azide, and 100 ml. normal saline. 16 ml. of AFP-agarose was mixed with 100 ul AFP-antisera at 50°C. The mixture was allowed to gel on a 8.2 x 10.1 cm. glass plate at 4°C, and then 42 3 mm. wells were punched in the gel. The wells were filled with 20 ul of fluid. Standards were used to fill the wells around the perimeter of the slide; in this way they not only served as known concentrations of AFP, but they also determined if the film of agar was uniform across the plate. The standards used were 3.14 ug/ml, 2.00 ug/ml, 1.04 ug/ml, and 0.43 ug/ml.

All amniotic fluid samples were initially diluted 1:5 with normal saline. All cloudy, blood-tinged, or meconium-stained fluids were centrifuged at 1700 rpm for 30 minutes to remove cellular debris before the dilutions were made. Every sample was assayed twice on a plate.

The plates were allowed to incubate in a moist environment at room temperature for 48 hours. In the manner first described by Alpert and co-workers[3] in 1970, the plates were then washed in saline for 24 hours and then placed in a 1% tannic acid solution for 15 minutes in order to highlight the precipitin rings.

Radial-immunodiffusion works on the principle that the antigen placed in the well will combine with an equivalent amount of antibody that is contained in the agar. When all of the antigen has been consumed, a ring of antigen-antibody complex will remain indefinitely, provided that the antiserum is incapable of redissolving the precipitin.

Mancini[20] proved a linear relationship between the concentration of antigen originally placed in the well and the area of the precipitin. Using that principle, the square of the diameters of the standards' precipitin rings were plotted against their known concentrations. The resulting graph was used to calculate the concentration of AFP in the unknowns.

Samples whose precipitin rings were larger than all of the standards were repeated at dilutions of 1:10 and 1:15. Fluids that failed to produce a ring, or whose ring was smaller than the smallest standard, were repeated undiluted.

In order to increase the sensitivity of the technique, agar plates were prepared using only 75 ul of antisera/16 ml of agar and 50 ul antisera/16 ml agar. In addition, new standards were prepared with 330 ng/ml, 210 ng/ml, 165 ng/ml, 132 ng/ml, and 110 ng/ml. Every sample that failed to produce a measurable ring in the initial assay was repeated undiluted with smaller concentration of antisera.

Evaluation of the Method

It was found that 16 ml of AFP agarose needed at least 50 ug antisera to yield readable precipitin rings. Using that concentration of antisera, it was possible to detect standards diluted as much as 1:400, or 165 ng/ml. At 1:500 dilution, or 133 ng/ml, no precipitin rings were seen. At dilutions greater than 1:150 (440 ng/ml) the slope of the dose-response curve began to flatten as the accuracy of the assay decreased and the limit of sensitivity was approached. By using the least possible concentration of antisera in the agar, the change in the slope was minimized. Figure 1 demonstrates the dose-response curve and the sensitivity and accuracy limits of the assay.

The with-in assay variability of the diameters of identical standards plated five times throughout a plate was 1.2%. All samples were plated in duplicate on each slide.

The between-assay variability was more complex. Results that could be read at 1:5 dilution were replated at the same dilution and gave a between-assay co-efficient of variation of 10%. Higher concentrations that were diluted 1:10 and 1:15 gave a variability of 9%. Small concentrations between 0.5 and 0.8 ug/ml had a co-efficient of variation of 13%. Samples with a mean concentration between 0.2 and 0.5 ug/ml, however, showed a standard deviation of $\pm$ 0.1 and a co-efficient of variation of 33%.

RESULTS

The AFP concentrations were assayed and recorded without prior knowledge of the identity of the sample. Gestational age was determined by ultrasound evaluation of biparietal diameter, and, where possible, the pediatrician's examination of the newborn. A graphic representation of the results is recorded in Figure 2.

37 control samples from the second trimester had a mean value of 7 ug/ml with a range of 2.4 to 17.5 ug/ml. 26 samples from gestational age 15-18 weeks had a mean concentration of 8.6 ug/ml, with a range of 2.6-17.5 ug/ml; the 11 samples from 19-22 weeks gestation ranged from 2.4-9.1 ug/ml with a mean value of 4.2 ug/ml. This range of normal AFP concentration is in agreement with the findings of other investigators using different assay techniques.[2,16,30]

Four amniotic fluid specimens from the second trimester were from pregnancies in which the fetus could be considered at risk for distress; all samples were found to have concentrations within the range of normal. Another specimen was from a fetal death complicated by Rh disease and trisomy 18. The AFP concentration was assayed to be 16.2 ug/ml which is well above the mean for that gestational age. Second trimester assays from abnormal pregnancies are summarized below.

Gestational Age	Complication	AFP ug/ml
21 weeks	methadone maintenance	2.7
18 weeks	diabetes	4.6
22 weeks	twins	5.2
18 weeks	meconium staining	6.5
19.5 weeks	Rh disease, 18^3, fetal death	16.2

Samples from the third trimester included specimens from two anencephalics in which the AFP concentration was over 20 ug/ml. The other 38 specimens were obtained after the 32nd week of gestation and all had AFP concentrations less than 0.8 ug/ml. The samples came from normal pregnancies and pregnancies complicated by diabetes (both Class A and B), Rh-disease, and pre-eclampsia.

With the exception of the anencephalics, all detectable levels in the third trimester samples were found to be between 165 and 800 ng/ml. The co-efficient of variation at this concentration was 33%; it was decided, therefore, to group together for statistical evaluation, all samples that yielded a visable precipitin ring yet had a concentration less than 800 ng/ml. All samples that, undiluted, failed to produce a definite precipitin ring on any plate were defined as having undetectable levels of alpha-fetoprotein, or an AFP concentration less than 165 ng/ml. The results are shown graphically in Figure 3.

The amniotic fluid samples taken from nine normal pregnancies between the 36th and 40th week of gestation all had concentrations of AFP less than 165 ng/ml. Also, a sample obtained at 42 weeks gestation

from an otherwise uncomplicated pregnancy had an undetectable AFP level.

Finding the concentrations of AFP at term in normal pregnancies to be under 165 ng/ml is in agreement with the work of Seppala and Ruoslahti.[28] Using radioimmunoassay, they found the AFP concentration at 37-38 weeks averaged 164 ng/ml; at 39-40 weeks the mean concentration was 115 ng/ml; and at 41-42 weeks, the level had decreased to 87 ng/ml.

18 samples from pregnancies stressed by Rh-disease, diabetes, and toxemia were obtained during the last four weeks of pregnancy. Only four of those specimens had undetectable levels of AFP. There was a highly significant correlation ($\chi^2=14.6$, $p<0.0005$) between the stress factors of diabetes, Rh-disease, and toxemia, and concentrations at term of AFP greater than 165 ng/ml.

Twelve of the fifteen samples from diabetics at term had detectable levels of AFP. Diabetes was shown to have a high correlation with increased levels of AFP ($\chi^2=14.4$, $p<0.0005$).

Six samples from Rh-immunized pregnancies were assayed, and all were found to have detectable levels of AFP. Five of the six, though, were obtained before the 36th week of gestation, and no normal samples were available for comparison. Two specimens from 34 and 35 weeks are of special note, however. They represented an infant that suffered from erythroblastosis fetalis, with 2+ jaundice at birth, 1-2+ edema,

Apgar scores of 3/6, and an initial hematocrit of 36. The infant was definitely stressed by its disease; however, AFP levels obtained one week prior to birth and at delivery were both 300 ng/ml. That AFP concentration is well within the range of normal seen by Seppala and Ruoslahti[28] in the 34-35 week gestational period.

This study, therefore, failed to show any relationship between Rh-immunization and elevated amniotic fluid AFP concentration. The investigation of Seppala and Ruoslahti[32] similarly failed to demonstrate a correlation between abnormal amniotic fluid AFP levels and Rh-immunization.

Five samples were obtained from women who had been treated for toxemia of pregnancy during the last trimester. Four out of five had concentrations of 500 ng/ml or greater, but two of the samples were obtained during the 34th and 35th weeks of gestation with no normal controls. Unfortunately, three samples obtained at term did not represent a sufficient volume to show a statistically significant correlation between elevated AFP levels and toxemia ($\chi^2 = 3.4$, $p < 0.07$).

Of particular interest is a sample from a 40-week gestation in a diabetic pre-eclamptic mother. The AFP concentration was assayed to be 800 ng/ml, the highest AFP level of any sample obtained at term. The infant involved was noted to suffer from transient hypoglycemia. The AFP level of 600 ng/ml at 36 weeks reflected a premature infant who was treated for prolonged hypoglycemia.

Three diabetics and one woman with Rh disease had more than one amniocentesis as term approached; they all showed AFP concentrations that decreased or remained constant with increasing gestation. The findings are summarized below.

Complication	Gestational Age	AFP ng/ml.
Diabetic Class A	37 weeks	400
	38	300
	39	300
Diabetic Class A	38 weeks	300
	39	Undetectable
Diabetic Class B	37 weeks	400
	38	300
Rh disease	34 weeks	300
	35	300

Eight serum samples were taken from women undergoing amniocentesis at term. All of the women had either Rh-disease or diabetes, and they all showed a serum level of 350 ng/ml (S. D. $\pm$ 100 ng/ml) which is within the range of normal for this gestational period according to the work of Seppala and Ruoslahti.[31]

COMMENT

Unfortunately, the radial-immunodiffusion technique is not sensitive enough to make a more detailed analysis of the AFP levels late in pregnancy. The low accuracy at concentrations less than 1.0 ug/ml made it impossible to compare groups with mildly elevated AFP levels. However, the assay does show a significant increase in amniotic fluid AFP in the face of diabetes and suggests an elevation in response to prolonged toxemia of pregnancy.

Alpha-fetoprotein is a major serum protein of the fetus and serves as a parameter of several aspects of fetal life. Elevated amniotic AFP concentration can reflect either increased fetal production of AFP or increased transudation of the protein. The high levels seen in anencephaly are an example of the latter.

Slightly but significantly elevated levels of AFP in association with maternal diabetes and pre-eclampsia cannot easily be explained by increased transudation. Norgaard-Pedersen and Klebe[23] demonstrated increased AFP levels in the serum of infants of diabetic mothers. Seppala and Ruoslahti[32] theorized that elevated amniotic levels might be the result of increased fetal production of the protein.

One can postulate that elevated AFP production results from delayed maturation of the fetal liver. Waldmann and McIntire[38] examined AFP

levels in patients with ataxia-telangiectasia. One of the theories to explain the disorder is that there is a defect in the interaction between the entoderm and mesoderm that is required for normal tissue differentiation, resulting in a failure of maturation of gut-associated organs. They found abnormally high serum levels of AFP in all patients with ataxia telangiectasia, suggesting an association between increased AFP and retarded differentiation.

Gitlin and Boesman[11] had demonstrated in the human fetus a direct relationship between an increase in the production of albumin and a turning off of the synthesis of AFP. Coid[7] demonstrated in mice a significant relationship between mild maternal infection, intrauterine growth retardation, and a low albumin/AFP ratio, indicating again that elevated AFP may be a reflection of neonatal immaturity in response to stress.

Two major theories have been suggested to describe the dynamics of AFP synthesis. These hypotheses have been proposed by cancer researchers to explain the production of AFP in hepatoma. Abelev[1] attributes the synthesis of AFP to the "hepatoblast," a specialized liver cell that predominates in fetal livers, and with maturity of the fetus is overrun by the hepatocyte.

Uriel[36] hypothesizes that there is only one type of liver cell. According to this theory, the genetic code for AFP production is turned

on during fetal life and is repressed with maturity. Under either view, the increased production of AFP reflects the presence of immature liver cells.

Zetterstrom and his colleagues[39] in 1958 described an increased frequency of non-hemolytic hyperbilirubinemia in 29 newborn infants of diabetic mothers. They hypothesized that the hyperbilirubinemia could reflect "immaturity-for-dates" in the infants.

Heringova's group,[15] however, compared the bilirubin clearance in normal newborns with the clearance rate in prematures and diabetics. They found that all groups had equal difficulty handling an increased bilirubin load on the first day of life; but on day five, both normals and diabetics rapidly secreted 100% of a test dose, whereas premature infants responded as sluggishly as they had on day one. They concluded that the hyperbilirubinemia seen in infants of diabetic mothers may be of different origin than that seen in premature infants.

Jersova and co-workers[18] were able to show a significant decrease in bilirubin excretion and choleresis in infant rats injected with serum from diabetic mothers as compared to rats injected with serum from normal pregnant women. They hypothesized that diabetic serum contains a factor that can inhibit choleresis and bilirubin excretion.

The mechanism responsible for the increased incidence of non-hemolytic hyperbilirubinemia in infants of diabetic mothers remains unclear. Perhaps the elevated AFP production in these infants is a parallel phenomenon and reflects liver immaturity.

It has been suggested that many of the clinical manifestations seen in infants of diabetic mothers can be explained by the following pathogenetic sequence:[25] maternal hyperglycemia, causing fetal hyperglycemia and hypertrophy of the fetal pancreatic islets, leading to fetal hyperinsulinism. This combination of hyperinsulinism and hyperglycemia results in increased hepatic glucose uptake and glycogen synthesis, accelerated lipogenesis, and augmented protein synthesis. This process, resulting in increased protein synthesis, may be the sole causative agent in the elevated levels of alpha-fetoprotein seen in these infants. However, this explanation fails to describe adequately why a fetal protein is elevated at term: perhaps the increased protein synthesis delays liver maturation, thereby elevating AFP production.

It has been demonstrated that alpha-fetoprotein is very useful diagnostically in fetal neural tube defects, and these data presented here suggest a possible correlation between fetal jeopardy in pregnancies complicated by diabetes and toxemia and increased concentrations of AFP. For late gestational analysis, the more complicated yet more sensitive radioimmunoassay would be helpful. A large study of RIA determined AFP levels at term in high-risk pregnancies has not been undertaken, and might help answer many questions about fetal development.

SUMMARY

Alpha-fetoprotein is known to be a useful diagnostic tool for early detection of fetal neural tube defects. Investigations have indicated that AFP can also be used to estimate gestational age and that abnormally elevated levels of AFP are often associated with fetal demise or fetal distress.

A radial immunodiffusion assay was used in this investigation to determine the AFP concentration in 80 amniotic fluid samples from the second and third trimesters of pregnancy. Anencephalic fetuses had amniotic fluid concentrations of AFP that were ten times normal. The levels of AFP in the second trimester were found to have a wide range of normal: 2-17.5 ug/ml. A sample from a fetal death at 20 weeks gestation had a concentration at the upper limit of normal.

The AFP level at term when the mother was diabetic was found to be significantly higher ($p < 0.0005$) than normal controls. Concentrations when the pregnancy was complicated by toxemia were elevated, but the difference was not statistically significant ($p < 0.07$). In cases of Rh immunization, the AFP levels were not found to be elevated.

The possible etiologies of increased AFP concentrations were discussed, and future experiments were suggested.

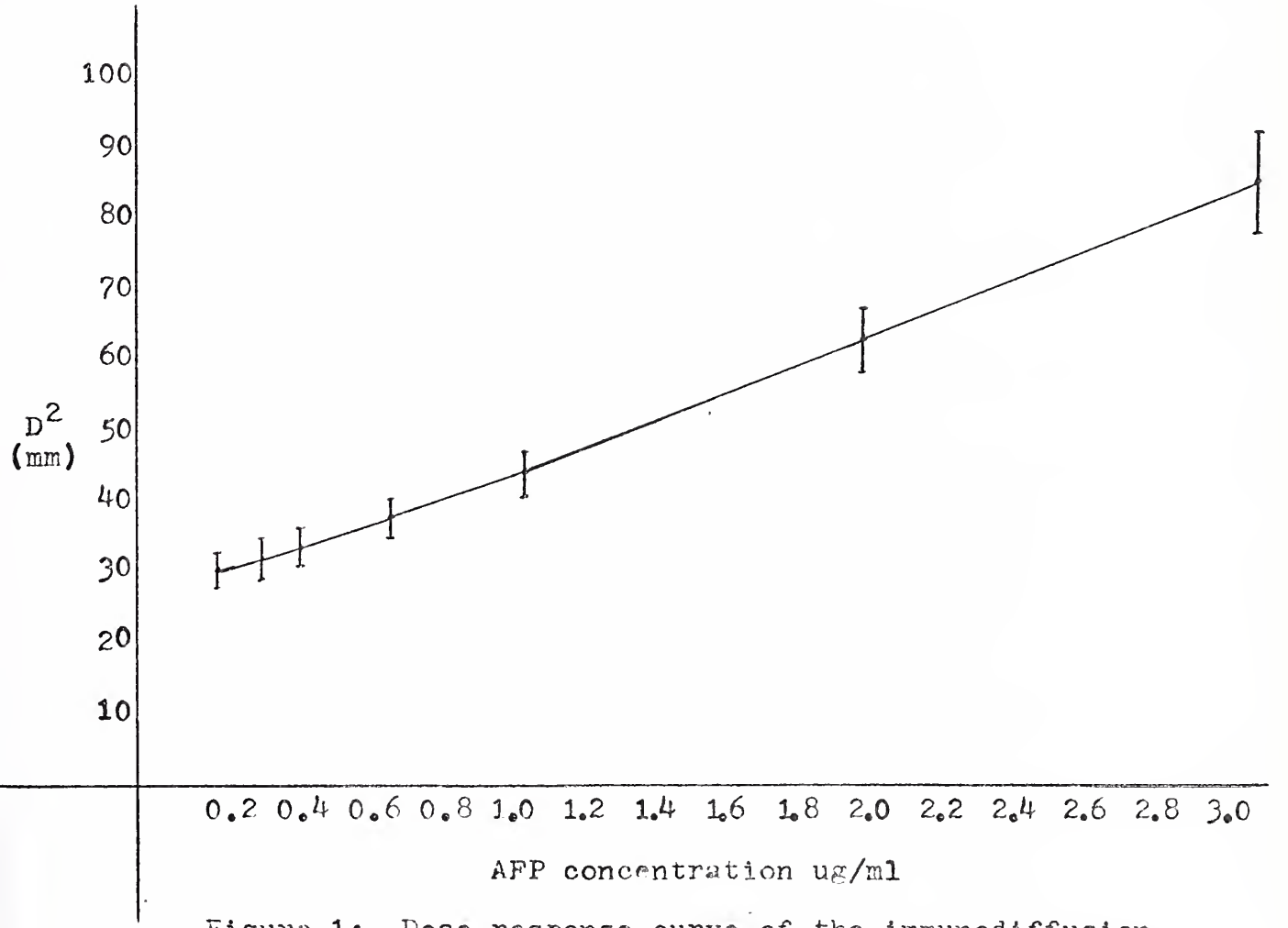

Figure 1: Dose response curve of the immunodiffusion assay for AFP with standard deviations graphed at different concentrations.

The slope of the curve is noted to flatten at concentrations less than 1 ug/ml. This dose-response curve demonstrates that at the lower limit of the assay, very small changes in the diameter of the precipitin ring result in significant changes in the estimated concentraion of AFP.

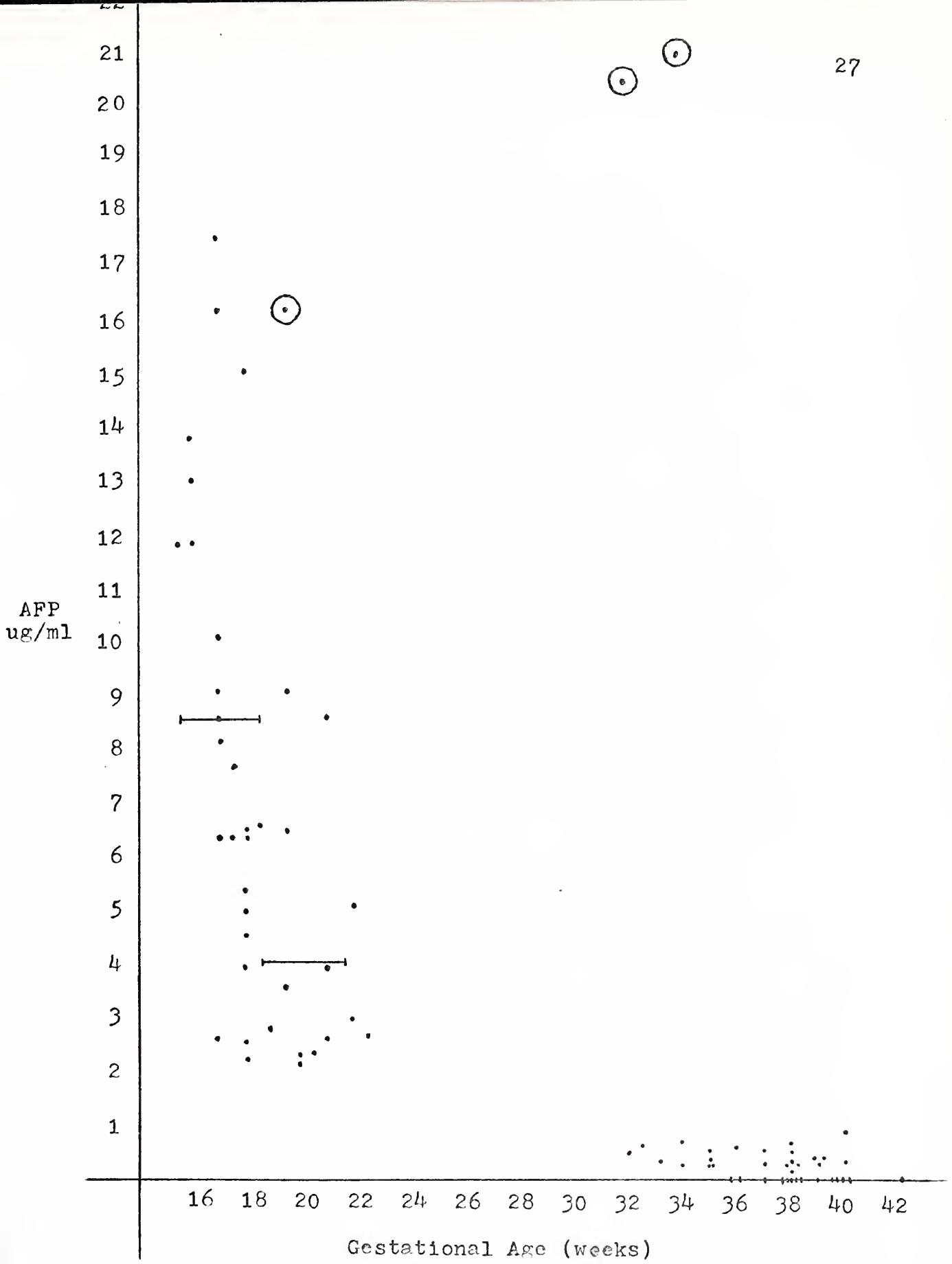

Figure 2: AFP concentrations at different gestational ages. Mean concentrations of AFP during the second trimester are represented by bars. Circled results represent fetal deaths.

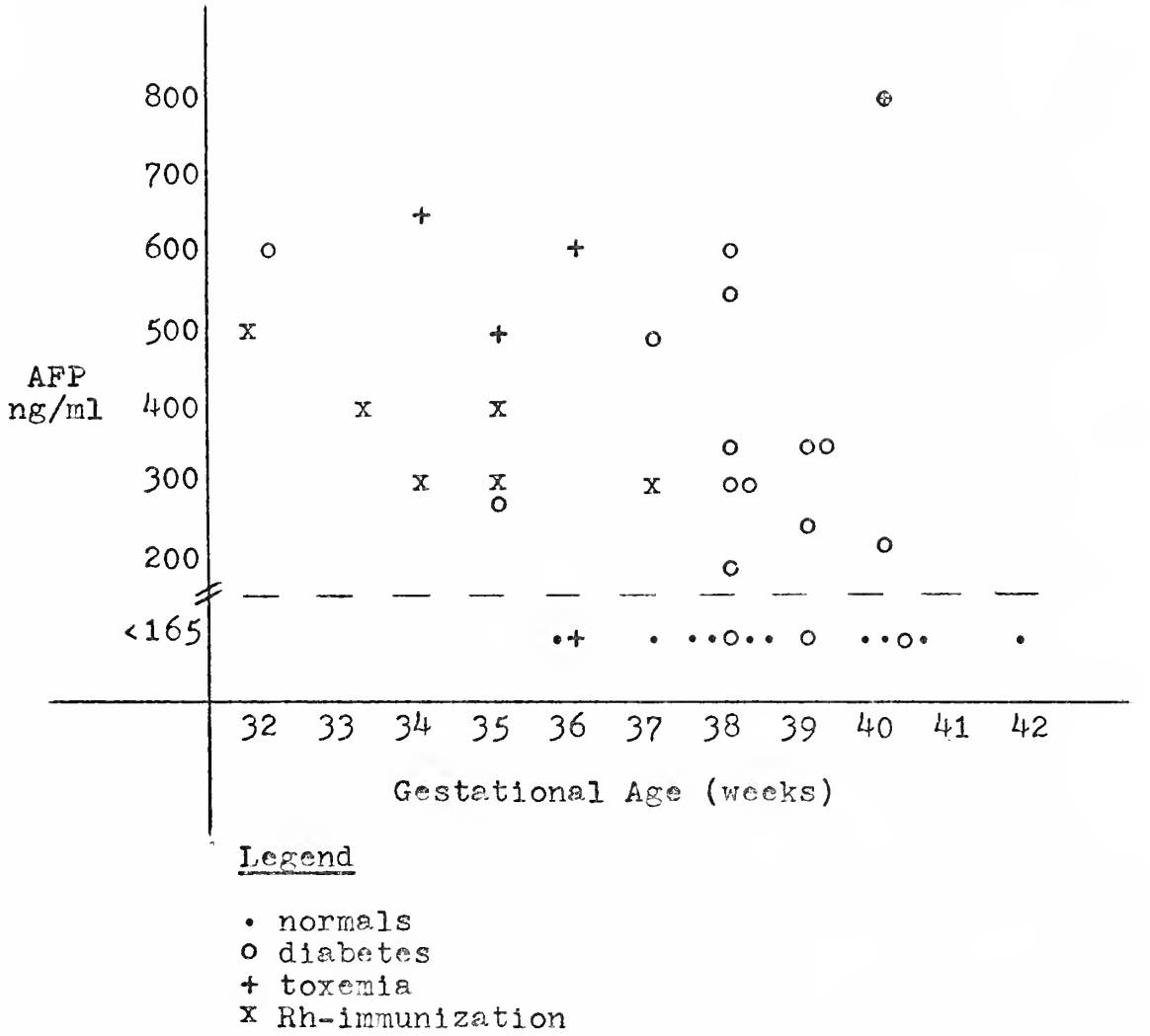

Figure 3: AFP concentrations in the third trimester

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